

Claims

1. A stably transformed plant, plant part, or plant cell culture containing in cell nuclei a heterologous DNA having a sequence encoding an RNA replicon operably linked or linkable to a transcription promoter,
wherein said sequence encoding an RNA replicon contains
 - (i) sequences for replicon function of said RNA replicon, said sequences being derived from a sequence of a plant RNA virus, and
 - (ii) a sequence of interest to be expressed from said RNA replicon,whereby said sequences for replicon function exhibit at selected localities of said sequence of said plant RNA virus function-conservative differences from said sequence of said plant RNA virus, said differences causing an increased frequency of replicon formation compared to an RNA replicon not exhibiting said differences.
2. The plant, plant part, or plant cell culture according to claim 1, wherein said function-conservative differences comprise a reduction of the deleterious effect in cell nuclei of a high A/U content in a transcript of said heterologous DNA on RNA replicon formation.
3. The plant, plant part, or plant cell culture according to claim 1 or 2, wherein said selected localities of said sequences of said plant RNA virus are localities of high A/U content.
4. The plant, plant part, or plant cell culture according to any one of claims 1 to 3, wherein said function-conservative differences comprise a reduction of a high A/U content in sequences for replicon function of said RNA replicon.
5. The plant, plant part, or plant cell culture according to claim 4, wherein said high A/U content is reduced by at least partial deletion or at least partial replacement by G/C bases.
6. The plant, plant part, or plant cell culture according to any one of claims 1 to 5, wherein said function-conservative differences comprise removal of cryptic splicing sites flanking A/U-rich regions.

7. The plant, plant part, or plant cell culture according to any one of claims 1 to 6, wherein said function-conservative differences comprise the insertion of one or more nuclear introns or one or more sequences capable of forming nuclear introns near or within A/U-rich localities of said sequences for replicon function.
8. The plant, plant part, or plant cell culture according to claim 7, wherein said sequence(s) capable of forming a nuclear intron are capable of forming a nuclear intron by site-specific recombination.
9. The plant, plant part, or plant cell culture according to any one of claims 1 to 8, wherein said sequence encoding an RNA replicon has one or more segments that code together for said RNA replicon, whereby formation of said RNA replicon requires rearrangement of said segments.
10. The plant, plant part, or plant cell culture according to any one of claims 1 to 9, wherein all cell nuclei of said plant contain said heterologous DNA.
11. The plant, plant part, or plant cell culture according to any one of claims 1 to 10, wherein said heterologous DNA is contained in a chromosome of said cell nuclei.
12. The plant, plant part, or plant cell culture according to claim 11, wherein all cells of said plant, plant part, or plant cell culture contain said heterologous DNA in a nuclear chromosome.
13. The plant, plant part, or plant cell culture according to one of claims 1 to 12, wherein said sequence encoding an RNA replicon further contains at localities of high A/T(U) content in said sequence of interest function-conservative differences causing an increased frequency of replicon formation compared to an RNA replicon not exhibiting said differences in said sequence of interest.
14. The plant, plant part, or plant cell culture according to any one of claims 1 to 13, wherein said plant RNA virus is a tobamovirus, preferably a tobacco mosaic virus.
15. The plant, plant part, or plant cell culture according to any one of claims 1 to 14, wherein said function-conservative differences are function-silent.

16. The plant part according to any one of claims 1 to 15, wherein said plant part is a seed.
17. A process of expressing a sequence of interest in a plant, plant part, or plant cell culture, comprising:
 - (a) providing a plant, plant part, or plant cell culture containing in cell nuclei a heterologous DNA having a sequence encoding an RNA replicon operably linked or linkable to a transcription promoter, wherein said sequence encoding an RNA replicon contains
 - (i) sequences for replicon function of said RNA replicon, said sequences being derived from a sequence of a plant RNA virus,
 - (ii) a sequence of interest,whereby said sequences for replicon function exhibit at selected localities of said sequences of said plant RNA virus function-conservative differences from said sequence of said plant RNA virus, said differences causing an increased frequency of replicon formation compared to an RNA replicon not exhibiting said differences; and
 - (b) causing expression of said sequence of interest.
18. The process according to claim 17, wherein said heterologous DNA is stably incorporated in a nuclear chromosome.
19. The process according to any one of claims 17 or 18, wherein said sequence of interest encodes a protein of interest and step (b) comprises causing expression of said protein of interest.
20. The process according to claim 19, wherein said expression of said protein of interest leads to production of a desired product in said plant, plant part or plant cells.
21. The process according to any one of claims 17 to 20, wherein said sequence encoding an RNA replicon has two or more segments that code together for said RNA replicon and step (b) comprises rearranging said segments such that said RNA replicon can be formed.
22. The process according to claim 21, wherein one of said segments is flanked by

recombination sites and said rearranging comprises site-specific recombination by a recombinase.

23. The process according to one of claims 21 to 22, wherein step (b) comprises providing a site-specific recombinase to said plant, plant part, or plant cell culture.
24. The process according to claim 23, wherein said site-specific recombinase is provided by transient transfection with a vector encoding said recombinase.
25. The process according to claim 23, wherein said site-specific recombinase is stably encoded in a nuclear chromosome under the control of a regulated promoter and step (b) comprises inducing said regulated promoter.
26. The process according to any one of claims 22 to 25, wherein said recombinase is selected from the group consisting of CRE, flippase, resolvase, FLP, SSV1-encoded integrase, R recombinase, phiC31 integrase, Inti integrase, phi 80, P22, P2, 186, and P4 recombinase, Tn3 resolvase, the Hin recombinase, the Cin recombinase, *E. coli* xerC and xerD recombinases, *Bacillus thuringiensis* recombinase.
27. The process according to any one of claims 22 to 26, wherein said recombinase is formed within plant cells from non-functional fragments of said recombinase by affinity interaction.
28. The process according to any one of claims 22 to 26, wherein said recombinase is formed within plant cells from non-functional fragments of said recombinase by intein-mediated protein trans-splicing.
29. The process according to one of claims 17 to 28, wherein said heterologous DNA or said sequence encoding said RNA replicon is linked to a regulated transcription promoter.
30. The process according to claim 29, wherein said regulated promoter is a chemically inducible promoter.
31. The process according to to any one of claims 17 to 30, wherein said providing of step (a) comprises transformation of a plant, plant part, or plant cells with a nucleic

acid molecule containing said heterologous DNA.

32. The process according to any one of claims 17 to 31, wherein said providing of step (a) comprises transient transformation of a plant, plant part, or cells of a plant cell culture with a nucleic acid molecule containing said heterologous DNA.
33. The process according to any one of claims 17 to 32, wherein step (a) is done by *Agrobacterium*-mediated transformation.
34. The process according to any one of claims 17 to 33, wherein step (a) comprises generating a transgenic plant stably transformed with said heterologous DNA.
35. A process of producing a transgenic plant stably transformed on a nuclear chromosome with a heterologous DNA as defined in claim 1, comprising transforming a plant or a plant part with a vector containing said heterologous DNA, selecting tissue of said plant containing on a nuclear chromosome said heterologous DNA, and regenerating a transgenic plant from said tissue.
36. A process of transiently expressing a sequence of interest in a plant, plant part, or plant cell culture, comprising:
transforming a plant, plant part, or plant cell culture with a heterologous DNA having a sequence encoding an RNA replicon operably linked or linkable to a transcription promoter, wherein said sequence encoding an RNA replicon contains
 - (i) sequences for replicon function of said RNA replicon, said sequences being derived from a sequence of a plant RNA virus,
 - (ii) a sequence of interest,whereby said sequences for replicon function exhibit at selected localities of said sequences of said plant RNA virus function-conservative differences from said sequence of said plant RNA virus, said differences causing an increased frequency of replicon formation compared to an RNA replicon not exhibiting said differences.
37. The process according to claim 36, wherein said transforming is performed by *Agrobacterium*-mediated transient transformation of T-DNA containing said heterologous DNA.

38. The process according to claim 36 or 37, wherein said transforming is performed by agroinfiltrating the stem and/or all leaves of *Nicotiana tabacum* plants.
39. The process according to any one of claims 17 to 38, wherein said function-conservative differences comprise a reduction of the deleterious effect in cell nuclei of a high A/U content in a transcript of said heterologous DNA on RNA replicon formation.
40. The process according to any one of claims 17 to 39, wherein said selected localities of said sequences of said plant RNA virus are localities of high A/U content.
41. The process according to any one of claims 17 to 40, wherein said function-conservative differences comprise a reduction of a high A/U content in sequences for replicon function of said RNA replicon.
42. The process according to claim 41, wherein said high A/U content is reduced by at least partial deletion or at least partial replacement by G/C bases.
43. The process according to any one of claims 17 to 42, wherein said function-conservative differences comprise removal of cryptic splicing sites flanking A/U-rich regions.
44. The process according to any one of claims 17 to 43, wherein said function-conservative differences comprise the insertion of one or more nuclear introns or one or more sequences capable of forming nuclear introns near or within A/U-rich localities of said sequences for replicon function.
45. The process according to claim 44, wherein said sequence(s) capable of forming a nuclear intron are capable of forming a nuclear intron by site-specific recombination.
46. The process according to any one of claims 17 to 45, wherein said sequence encoding an RNA replicon has one or more segments that code together for said RNA replicon, whereby formation of said RNA replicon requires rearrangement of said segments.
47. The process according to any one of claims 17 to 46, wherein all cell nuclei contain

said heterologous DNA.

48. The process according to one of claims 17 to 47, wherein said sequence encoding an RNA replicon further contains at localities of high A/T(U) content in said sequence of interest function-conservative differences.
49. The process according to any one of claims 17 to 48, wherein a selected locality is identified by
 - (A) transforming a plant, plant part, or plant cell culture with a heterologous DNA as defined in claim 17 but lacking said function-conservative differences,
 - (B) performing RT-PCR on RNA derived in said plant, plant part, or plant cell culture from said heterologous DNA of step (A) and sequencing the product of said RT-PCR, and
 - (C) identifying in the sequence of said product of said RT-PCR a selected locality as a locality of an undesired splicing event.
50. The process according to any one of claims 17 to 49, wherein said plant RNA virus is a tobamovirus, preferably a tobacco mosaic virus.
51. The process according to any one of claims 17 to 50, wherein said function-conservative differences are function-silent.
52. The process according to any one of claims 17 to 51, wherein said plant, plant part or plant cell culture contains two or more different heterologous DNAs and said process comprises formation of two different RNA replicons from which two different proteins of interest are co-expressed.
53. The process according to any one of claims 17 to 52, wherein said RNA replicon is capable of exhibiting an increased frequency of replicon formation in a plant, plant part or plant cell culture other than the natural host plant of the RNA virus from which said RNA replicon is derived.
54. The process according to any one of claims 17 to 53, wherein said plant, plant part, or plant cell culture is provided with two different heterologous DNAs each having a sequence of interest, whereby two different sequences of interest are expressed.

55. The process according to any one of claims 17 to 54, wherein said transforming of said plant, plant part, or plant cell culture is done by infiltrating said plant, plant part, or plant cell culture with a suspension of *Agrobacteria*, said suspension having a concentration corresponding to a calculated optical density at 600 nm of at most 0.04, preferably at most 0.01, more preferably at most 0.004, and most preferably at most 0.001, whereby said calculated optical densities are defined by an at least 25-fold, preferably at least 100-fold, more preferably at least 250-fold, and most preferably at least 1000-fold dilution, respectively, of a suspension of said *Agrobacteria* of an OD at 600 nm of 1.0.
56. A process of expressing a sequence of interest in a plant, plant part, or plant cell culture, comprising:
transforming a plant, plant part, or plant cell culture with a suspension of *Agrobacteria*, said *Agrobacteria* containing in T-DNA a heterologous DNA having a sequence encoding a replicon operably linked or linkable to a transcription promoter, wherein said sequence encoding a replicon contains
- (i) sequences for replicon function of said replicon, said sequences being derived from a sequence of a plant virus,
 - (ii) a sequence of interest,
- whereby said suspension of *Agrobacteria* has a concentration of cells of said *Agrobacteria* corresponding to a calculated optical density at 600 nm of at most 0.04, preferably at most 0.01, more preferably at most 0.004, and most preferably at most 0.001, whereby said calculated optical densities are defined by an at least 25-fold, preferably at least 100-fold, more preferably at least 250-fold, and most preferably at least 1000-fold dilution, respectively, of a suspension of said *Agrobacteria* of an OD at 600 nm of 1.0.
57. Nucleic acid molecule containing a DNA sequence encoding an RNA replicon operably linked or linkable to a transcription promoter, wherein said sequence encoding an RNA replicon contains
- (i) sequences for replicon function of said RNA replicon, said sequences being derived from a sequence of a plant RNA virus,
 - (ii) a sequence of interest to be expressed from said RNA replicon,
- whereby said sequences for replicon function correspond to sequences of said plant RNA virus and exhibit at selected localities of said sequences of said plant RNA virus function-conservative differences from said sequence of said plant RNA virus, said

differences being capable of causing an increased frequency of replicon formation compared to an RNA replicon not exhibiting said differences, when said nucleic acid molecule is introduced in plant cells or a plant.